**Supplementary Table. 1 The applications of *in vivo* 3PM imaging**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Species | Animal | Region | Depth (μm) | Sample type | Label methods | Marker | Ref. |
| Mouse | FVB/N mouse |  | 1300 | vasculature | injection | Texas-Red–dextran | (Horton et al., 2013) |
|  | Thy1-Brainbow1.0 |  | 1060-1120 | pyramidal neuron | transgenic mice | RFP |
|  | CaMKII-tTA/tetO-GCaMP6s | Hippocampal stratum pyramidale |  | CaMKII+ neuron | transgenic mice | GCaMP6s | (Li et al., 2020; Ouzounov et al., 2017; Wang et al., 2018) |
|  | Thy1-jRGECO1a | Cortex | 750 | Thy1+ neuron | transgenic mice | jRGECO1a | (Li et al., 2020) |
|  | Thy1-YFP-H | Cortex | 747-767 | Thy1+ neuron | transgenic mice | YFP | (Rodríguez et al., 2021) |
|  |  | Hippocampus | 719 | Thy1+ neuron | transgenic mice | YFP |
|  |  | Dorsal horn of the spinal cord | 400 | neuron | AAV8-Syn-jGCaMP7s | jGCaMP7s |
|  | Thy1-EGFP(M) | Cortex | 1450 | Thy1+ neuron | transgenic mice | EGFP | (Streich et al., 2021) |
|  |  | Hippocampus |  | Thy1+ neuron | transgenic mice | EGFP |
|  | Gad2-Cre::Ai14 | Hippocampus |  | Gad2+ neuron | transgenic mice | tdTomato |
|  |  | Corpus callosum | 1037 | Fibrous astrocyte | AAV2/5-GFAP-GCaMP6s | GCaMP6s |
|  |  | Corpus callosum | 862  | Fibrous astrocytes | AAV2/5-GFAP-GCaMP6s | GCaMP6s |
|  |  | Layer 6 of the visual cortex | 835 | Protoplasmic astrocyte | AAV2/5-GFAP-GCaMP6s | GCaMP6s |
|  |  | Cortex | 784 | protoplasmic astrocyte | AAV2/5-GFAP-GCaMP6f | GCaMP6f |
|  | CCK-Cre::Ai162D | Somatosensory cortex |  | CCK+ neuron | transgenic mice | GCaMP6s | (Qin et al., 2022) |
|  | Scnn1a-Cre | Visual cortex | -- | sodium channel, nonvoltage-gated 1 alpha+ neuron (cortex layer 4) | AAV1/2-hSyn-FLEX-jGCaMP7f | jGCaMP7f | (Klioutchnikov et al., 2022) |
|  | Ntsr1-cre | Visual cortex | -- | neurotensin receptor 1+ neuron (cortex layer 6) | AAV1/2-hSyn-FLEX-jGCaMP7f | jGCaMP7f |
|  | Cx3Cr1-GFP | cortex |  | microglia | transgenic mice | GFP |
|  | CD1 | White matter and SVZ | 1150–1472 | RGC like neural Neural stem cell | microinjection | CTO | (Sun et al., 2022) |
|  |  | SVZ | 1386 | Neuroblasts | microinjection | CTO |
|  |  | OB | 1050 | Neural stem cell | microinjection | CTO |
|  |  | SVZ and LV |  | Neural stem cell | microinjection | Cell Tracker Green |
|  | Nestin-CreER2::Ai9 | SVZ | 1123 | Neural stem cell | transgenic mice | tdTomato |
|  |  | Popliteal lymph nodes | 600–900 | vasculature | injection | fluorescein; Alexa Fluor 647 |
|  |  | LPS-induced inflamed LNs |  | CD8+ T cell; CD4+ T cell | injection | CMRA; CFSE | (Choe et al., 2022) |
|  | Cγ1Cre-confetti mouse | Popliteal lymph nodes |  | GC B cell | transgenic mice | CFP; GFP; YFP; RFP |  |
|  |  | Popliteal lymph nodes |  | blood vessel | injection | Alexa-Fluor-647 |  |
| Rat |  |  |  |  |  |  |  |
|  | Lister Hooded rats | posterior parietal cortex; visual cortex neurons | 1120 | neuron | AAV1/2-Syn-GCaMP6s-WPRE | GCaMP6s | (Klioutchnikov et al., 2020) |
| Zebrafish |  |  |  |  |  |  |  |
|  | Tg(vglut2a:loxP-DsRed-loxP-GFP |  |  |  | transgenic animal | DsRed, GFP | (Chow et al., 2020) |
|  | Tg(vglut2a:mtdtomato) |  |  |  | transgenic animal | tdTomato |  |
|  | Tg(vglut2a:EGFP) | the stratum periventriculare in the optic tectum | 750 | glutamatergic neuron | transgenic animal | EGFP |  |
|  |  | entire Telencephalon | 1,045 | elavl3 promoter | transgenic animal | GCaMP6s |  |
|  |  | Cerebellar | 700–750 | Purkinje and granule cell | transgenic animal | GFP |  |
|  | Tg (kdrl:EGFP) |  |  | vascular epithelial cells | transgenic animal | EGFP | (Chen et al., 2018) |
| Drosophila |  |  |  |  |  |  |  |
|  |  | Mushroom body |  | Kenyon cell | transgenic animal | mCherry or jRCaMP1b | (Aragon et al., 2022) |
|  |  | Mushroom body |  | Kenyon cell | transgenic animal | GFP or GCaMP6s |
|  | GH146>GCaMP5 strain | Antenna lobe |  | excitatory projection neurons | transgenic animal | GCaMP5 | (Chen et al., 2018) |